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PATENT
Docket No. 300622004620

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Nora Durant
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Rajesh GOKHALE et al.

Serial No.: 10/091,244

Filing Date: 04 March 2002

For: METHODS TO MEDIATE
POLYKETIDE SYNTHASE MODULE
EFFECTIVENESS

Examiner: To be assigned

Group Art Unit: 1652

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL
APPLICATION

BOX MISSING PARTS
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is in response to the Notice to File Missing Parts of Nonprovisional Application mailed April 25, 2002, for which a response was due on June 25, 2002. Accordingly, a one month petition for extension of time is filed herewith, extending the time for response to July 25, 2002.

Please enter the following sequence listing, amendments and remarks.

07/30/2002 HBERHE 00000070 031952 10091244
02 FC:215 55.00 CH

sd-96685

In the Sequence Listing:

Please insert the attached paper copy of the Sequence Listing as new pages 1-7 in the above-captioned application. A computer-readable form (CRF) copy of the Sequence Listing accompanies this response.

AMENDMENT

In the Specification:

Please replace paragraph [00113] with the following rewritten paragraph [00113]:

--The N-terminal linker of M3 was synthesized by New England Peptide (Fitchburg, MA). The peptide sequence was as follows, M3 N-term: H₂N-MTDSEKVAEYLRRATLDLRAARQRIRELESD-amide. (SEQ ID NO: 20)--

Please replace paragraph [00114] with the following rewritten paragraph [00114]:

--**Construction of Plasmids.** Plasmid pBP19 contains module 2 of DEBS (M2) and is a derivative of pRSG64 (Gokhale, R. S., *et al.*, (1999) *Science* 284, 482-485), where the thioesterase domain was replaced with a *SpeI-EcoRI* fragment containing the natural C-terminal linker for module 2 to make pBP19. Plasmid pST179 encodes a derivative of M2 containing the C-terminal linker of DEBS module 4 (M4). The C-terminal linker of M4 was obtained as a *SpeI-EcoRI* fragment by PCR using the primers 5'-ACT AGT **AGG CTG TTC GCG GCC TCA C**-3' (SEQ ID NO: 21) and 5'-G GGA ATT **CAG GTC CTC TCC CCC GC**-3' (SEQ ID NO: 22) (bold sequences complement DEBS sequence). The PCR amplicon was inserted after M2 using the engineered sites, yielding pST179. This plasmid, pRSG34, encodes module 3 of DEBS (M3) with its own N-terminal linker and with the thioesterase fused to the C-terminus. Its construction has been described previously (*id.*). Plasmid pST132 encodes a derivative of M3 + TE, where the natural N-terminal linker of pRSG34 has been replaced with the N-terminal linker of module

--**Construction of Plasmids.** The gene encoding ACP4(4) was amplified as an *NdeI*-*EcoRI* PCR fragment (523 bp) using the primers 5'-CCATATGGTGGTTCGACCGGCTCG-3' (SEQ ID NO: 23) and 5'-GAATTCCTA-CAGGTCCTCTCCCC-3' (SEQ ID NO: 24) (sequences complementary to DEBS shown in bold). The PCR product was cloned into pET28a (Novagen) to yield plasmid pNW8. Plasmid pST157 encodes a bimodular fusion between module 1 of DEBS1 and module 5 of DEBS3, with the thioesterase domain fused downstream of module 5 ("M1+M5+TE"). This fusion, which was engineered by taking advantage of the natural, conserved *BsaBI* sites located at the start of the KS domains of modules 2 and 5, also includes the loading didomain of DEBS1. The "linker" sequence that covalently bridges the fused modules is the natural sequence between modules 1 and 2, as in DEBS1. The fusion junction between module 5 and the thioesterase domain is identical to that in plasmid pRSG46.²³ Similarly, plasmid pST92 encodes an "M1+M6+TE" bimodular fusion. Its construction, which is completely analogous to that of pST157, involves introduction of this bimodular PKS gene from pST96, Gokhale, et al., *Science* 1999, 284, 482-485, as an *NdeI*-*EcoRI* into pET-21c (Novagen). The construction of genes encoding (5)M2+TE, (3)M3+TE, (5)M5+TE, and (5)M6+TE (pRSG64, pRSG64, pRSG46, and pRSG54, respectively) have been described previously, *id.*, as well as the construction of a gene encoding (5)M3+TE (pST132). See Tsuji, et al., *Biochemistry* 2001.--

REMARKS

The Specification has been amended to include sequence identification numbers which were omitted at the time of filing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made.".


The undersigned hereby states that the paper copy of the Sequence Listing and the computer readable form copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.825(a) and (b), respectively, are the same and contain no new matter. Accordingly, entry of the Sequence Listing into the above-captioned case is respectfully requested.

In the unlikely event that the patent office determines that extensions and/or other relief is required, applicant petition for any required relief including extensions of time and authorize the assistant commissioner to charge the cost of such petitions and/or fees due to our deposit account no. 03-1952 under order no. 300622004620. The assistant commissioner is not authorized to charge the cost of the issue fee to the deposit account.

Respectfully submitted,

Dated: July 24, 2002

By:


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph [00113] at page 31 has been amended as follows:

The N-terminal linker of M3 was synthesized by New England Peptide (Fitchburg, MA). The peptide sequence was as follows, M3 N-term: H₂N-MTDSEKVAEYLRRATLDLRAARQRIRELESD-amide. (SEQ ID NO: 20)

Paragraph [00114] at page 32 has been amended as follows:

Construction of Plasmids. Plasmid pBP19 contains module 2 of DEBS (M2) and is a derivative of pRSG64 (Gokhale, R. S., *et al.*, (1999) *Science* 284, 482-485), where the thioesterase domain was replaced with a *SpeI-EcoRI* fragment containing the natural C-terminal linker for module 2 to make pBP19. Plasmid pST179 encodes a derivative of M2 containing the C-terminal linker of DEBS module 4 (M4). The C-terminal linker of M4 was obtained as a *SpeI-EcoRI* fragment by PCR using the primers 5'-ACT AGT **AGG CTG TTC GCG GCC TCA C**-3' (SEQ ID NO: 21) and 5'-G GGA ATT **CAG GTC CTC TCC CCC GC**-3' (SEQ ID NO: 22) (bold sequences complement DEBS sequence). The PCR amplicon was inserted after M2 using the engineered sites, yielding pST179. This plasmid, pRSG34, encodes module 3 of DEBS (M3) with its own N-terminal linker and with the thioesterase fused to the C-terminus. Its construction has been described previously (*id.*). Plasmid pST132 encodes a derivative of M3 + TE, where the natural N-terminal linker of pRSG34 has been replaced with the N-terminal linker of module 5 of DEBS (M5). This substitution required the replacement of the *NdeI-BsaBI* fragment of pRSG34 with the corresponding fragment from pJRJ10 (Jacobsen, J. R., *et al.*, (1998) *Biochemistry* 37, 4928-4934). All constructs were cloned into pET-21c (Novagen) vectors for expression in *Escherichia coli*.

Paragraph [00131] at page 40 has been amended as follows:

Construction of Plasmids. The gene encoding ACP4(4) was amplified as an *NdeI*-*EcoRI* PCR fragment (523 bp) using the primers 5'-CCATATGGTGGTCGACCGGCTCG-3' (SEQ ID NO: 23) and 5'-GAATTCCTA-CAGGTCCTCTCCCCC-3' (SEQ ID NO: 24) (sequences complementary to DEBS shown in bold). The PCR product was cloned into pET28a (Novagen) to yield plasmid pNW8. Plasmid pST157 encodes a bimodular fusion between module 1 of DEBS1 and module 5 of DEBS3, with the thioesterase domain fused downstream of module 5 ("M1+M5+TE"). This fusion, which was engineered by taking advantage of the natural, conserved *BsaBI* sites located at the start of the KS domains of modules 2 and 5, also includes the loading didomain of DEBS1. The "linker" sequence that covalently bridges the fused modules is the natural sequence between modules 1 and 2, as in DEBS1. The fusion junction between module 5 and the thioesterase domain is identical to that in plasmid pRSG46.²³ Similarly, plasmid pST92 encodes an "M1+M6+TE" bimodular fusion. Its construction, which is completely analogous to that of pST157, involves introduction of this bimodular PKS gene from pST96, Gokhale, et al., *Science* 1999, 284, 482-485, as an *NdeI*-*EcoRI* into pET-21c (Novagen). The construction of genes encoding (5)M2+TE, (3)M3+TE, (5)M5+TE, and (5)M6+TE (pRSG64, pRSG64, pRSG46, and pRSG54, respectively) have been described previously, *id.*, as well as the construction of a gene encoding (5)M3+TE (pST132). See Tsuji, et al., *Biochemistry* 2001.